

Claims:

We hereby claim:

1. A non-naturally occurring glycolipopeptide comprising at least five amino acids, at least one amino acid being a glycosylated amino acid and at least one amino acid being a lipidated amino acid, where at least one lipidated amino acid is an interior amino acid, said glycolipopeptide comprising at least one disease-associated epitope.

2. The glycolipopeptide of claim 1 where at least one epitope is a cancer-associated epitope.

3. The glycolipopeptide of claim 1 where at least one epitope is a bacteria-associated epitope.

4. The glycolipopeptide of claim 1 where at least one epitope is a parasite -associated epitope.

5. The glycolipopeptide of claim 1 where at least one epitope is a virus-associated epitope.

6. The glycolipopeptide of claim 2 where at least one epitope is a MUC1 epitope.

7. The glycolipopeptide of any one of claims 1-6 which comprises at least one B cell epitope.

8. The glycolipopeptide of any one of claims 1-6 which comprises at least one T cell epitope.

9. A non-naturally occurring glycolipopeptide comprising at least five amino acids, at least one amino acid being a glycosylated amino acid and at least one amino acid being a lipidated amino acid, said glycolipopeptide comprising at least one B cell epitope or at least one T cell epitope, at least one of said epitopes being a MUC1 cancer-associated epitope.

10. The glycolipopeptide of any one of claims 1-9 which comprises at least one MUC1 B cell peptide epitope and at least one MUC1 T cell peptide epitope.

11. The glycopeptide of any one of claims 1-10 which comprises the amino acid sequence PDTRP (AAs 6-10 of SEQ ID NO:10).

12. The glycolipopeptide of claim 11 which comprises the amino acid sequence SAPDTRP (AAs 4-10 of SEQ ID NO:10).

13. The glycolipopeptide of any one of claims 1-9 which comprises at least one copy of (a) the MUC1 consensus tandem repeat

GVTSAPDTRPAPGSTAPPAH (SEQ ID NO:10) ,

(b) a cyclic permutation thereof, or (c) a sequence substantially identical to (a) or (b) above.

14. The glycolipopeptide of claim 13 which comprises at least two copies of (a), (b) or (c).

15. The glycolipopeptide of any one of claims 1-14 where at least one glycosylated amino acid is O-glycosylated.

16. The glycolipopeptide of any one of claims 1-15 where at least one glycosylated amino acid is N-glycosylated.

17. The glycolipopeptide of any one of claims 1-16 where at least one glycosylated amino acid is S-glycosylated.

18. The glycolipopeptide of any one of claims 1-17 which comprises a tumor-associated carbohydrate epitope.

19. The glycolipopeptide of claim 18 where the carbohydrate epitope is GalNAc (Tn).

20. The glycolipopeptide of claim 18 where the carbohydrate epitope is sialyl Tn.

21. The glycolipopeptide of claim 18 where the carbohydrate epitope is Gal-GalNAc (TF).

5 22. The glycolipopeptide of claim 11 where the threonine of PDTRP is glycosylated.

23. The glycolipopeptide of claim 22 where the threonine of PDTRP is O-linked to Tn.

10 24. The glycolipopeptide of any one of claims 1-23 where at least two amino acids are glycosylated.

25. The glycolipopeptide of claim 9 in which at least one interior amino acid is lipidated.

26. The glycopeptide of any one of claims 1-25 in which at least two amino acids are lipidated.

15 27. The glycolipopeptide of any one of claims 1-25 in which at least two interior amino acids are lipidated.

28. The glycolipopeptide of any one of claims 1-27 in which all of the lipidated amino acids are interior amino acids.

20 29. The glycolipopeptide of any one of claims 1-28 characterized by a carboxy terminal sequence SSL, where each of the serines is lipidated.

30. The glycolipopeptide of any one of claims 1 to 29 in which there are not more than 200 amino acids.

25 31. The glycolipopeptide of any one of claims 1 to 29 in which there are not more than 50 amino acids.

30 32. The glycolipopeptide of any one of claims 1-31 wherein at least one lipidated amino acid comprises a strongly lipophilic group comprising at least 6 atoms other than hydrogen.

33. The glycolipopeptide of any one of claims 1-31 wherein at least one lipidated amino acid comprises a

strongly lipophilic group comprising at least 11 atoms other than hydrogen.

34. The glycolipopeptide of any one of claims 1-31 wherein at least one lipidated amino acid comprises a strongly lipophilic group comprising at least 13 atoms other than hydrogen.

35. The glycolipopeptide of any one of claims 1-31 wherein at least one lipidated amino acid comprises a strongly lipophilic group comprising at least 21 atoms other than hydrogen.

36. The glycolipopeptide of any one of claims 1-35 where said group consists of not more than 100 atoms other than hydrogen.

37. The glycolipopeptide of any one of claims 1-35 where said group consists of not more than 40 atoms other than hydrogen.

38. The glycopeptide of any one of claims 1-37 in which at least one strongly lipophilic group of at least one lipidated amino acid has a logP, as predicted by the Meylan algorithm of at least 2.7.

39. The glycopeptide of claim 38 where said predicted logP is at least 3.

40. The glycopeptide of claim 38 where said predicted logP is at least 4.

41. The glycopeptide of claim 38 where said predicted logP is at least 5.

42. The glycopeptide of claim 38 where said predicted logP is at least 6.

43. The glycopeptide of claim 38 where said predicted logP is at least 7.

44. The glycopeptide of claim 38 where said predicted logP is at least 8.

45. The glycopeptide of claim 38 where said predicted logP is at least 9.

46. The glycopeptide of claim 38 where said predicted logP is at least 10.

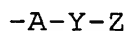
5 47. The glycolipopeptide of any one of claims 1-46 in which the amino terminal amino acid comprises a strongly lipophilic group.

48. The glycolipopeptide of any one of claims 1-47 in which the carboxy terminal amino acid comprises a strongly
10 lipophilic group.

49. The glycolipopeptide of any one of claims 1-48 in which at least one lipidated amino acid is selected from the group consisting of lipidated Ser, Thr, Asp, Glu, Cys, Tyr, Lys, Arg, Asn or Gln.

15 50. The glycolipopeptide of claim 49 where the lipidated amino acid is lipidated Ser or Thr.

51. The glycolipopeptide of any one of claims 1-50 where at least one lipidated amino acid comprises a side chain of the formula



where A is optional but, if present, is an organic group of not more than 12 atoms other than hydrogen;
Y is a spacer of not more than 12 atoms other than hydrogen, and comprising nitrogen, oxygen, sulfur or phosphorous, and
25 Z is a strongly lipophilic group.

52. The glycolipopeptide of claim 51 in which A, if present, is an alkyl of 1-4 carbon atoms.

53. The glycolipopeptide of claim 52 in which A is present and is $-CH_2-$ or $-CH(CH_3)-$.

30 54. The glycolipopeptide of any one of claims 1-53 in which Y comprises a group selected from the group consisting

of -O-, -S-, -NH-, -NR-, -PO₄-, -C(=O)-, -C(=S)-, -C(=NH)-, and -C(=NR)-, where R is 1-4 alkyl.

55. The glycolipopeptide of claim 54 in which Y is -NHCO-, -OCO- or -SCO-.

5 56. The glycolipopeptide of claim 54 in which Y is -CONH- or -CH₂NH-.

57. The glycolipopeptide of claim 54 in which Y is -O-, -S- or -NH-.

10 58. The glycolipopeptide of any one of claims 51-57 in which -Y-Z is itself a strongly lipophilic group.

59. The glycolipopeptide of claim 58 in which A is present and -A-Y-Z is itself a strongly lipophilic group.

60. The glycolipopeptide of any one of claims 51-59 in which Z is at least partially aromatic.

15 61. The glycolipopeptide of any one of claims 51-59 in which Z is aliphatic.

20 62. The glycolipopeptide of any one of claims 51-61 in which Z comprises at least one moiety of the form -A'-Y'-Z', where A', Y' and Z' are defined analogously to A, Y and Z, respectively.

63. The glycolipopeptide of claim 62 where Y' is -O- and Z' is an alkyl group.

25 64. The glycolipopeptide of claim 63 where A is - (CH₂)_i-, where i is 0 or 1, or Z' is -(CH₂)_jCH₃, where j is 6 to 26.

30 65. The glycolipopeptide of any one of claims 62-64 in which Z comprises -B(-Y'-Z')_n, in which B is a branched organic group of not more than 12 atoms than hydrogen, each Y' is an independently chosen spacer of not more than 12 atoms other than hydrogen, and comprising nitrogen, oxygen, sulfur or phosphorous, and each Z' is an independently chosen strongly lipophilic group, and n is at least two.

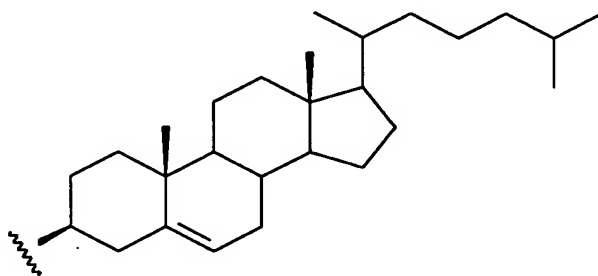
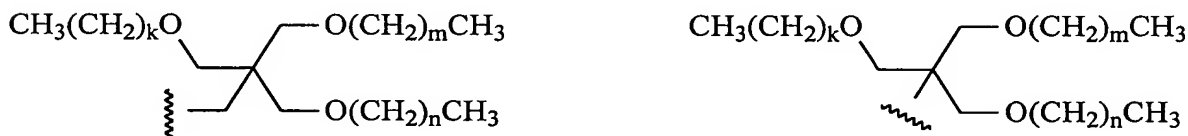
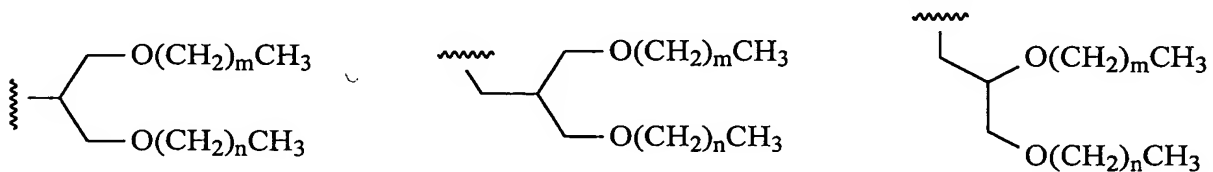
66. The glycolipopeptide of claim 65 in which n is 2 or 3.

67. The glycolipopeptide of claims 65 or 66 in which each Y' is $-O-$ and each Z' independently is $-(CH_2)_jCH_3$, where $j=6$ to 26.

68. The glycolipopeptide of claim 67 in which $n=2$ and B is $-\text{CH}(\text{CH}_2-)_2$.

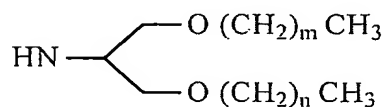
69. The glycolipopeptide of claim 67 in which $n=2$ and B is $-\text{C}(\text{CH}_2-)_3$.

70. The glycolipopeptide of claim 1 where the strongly lipophilic group of at least one lipidated amino acid comprises at least one of the following structures:



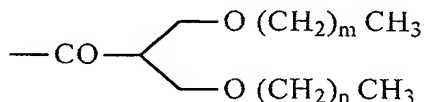
where m , n , and k are independent integers with values ranging from 3 to 30.

5 71. The glycolipopeptide of claim 1 where at least one
lipidated amino acid comprises the structure



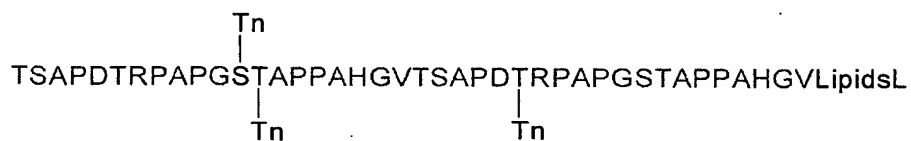
where m and n are independent integers with values ranging
10 from 6 to 26.

72. The glycolipopeptide of any one of claims 1-7 where the strongly lipophilic group at least one lipidated amino acid comprises the structure



15 where m and n are independently chosen integers 6 to 26.

73. The glyco-lipo-peptide of claim 1, having the following structure:



where **Tn** is a N-acetyl galactosamine, and "Lipids" refers to
20 two or more consecutive lipidated amino acids.

74. The glyco-lipo-peptide of claim 1, having the following structure:



75. A method of eliciting an immune response which comprises administering an effective amount of a glycolipopeptide according to any one of claims 1-74 to a subject.

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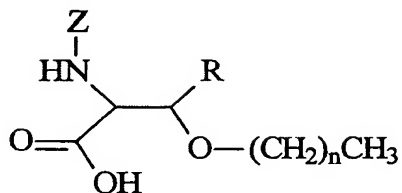
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81. A method of eliciting an immune response which comprises administering an effective amount of a composition according to any one of claims 77-80 to a subject.

82. A lipidated amino acid of the following formula



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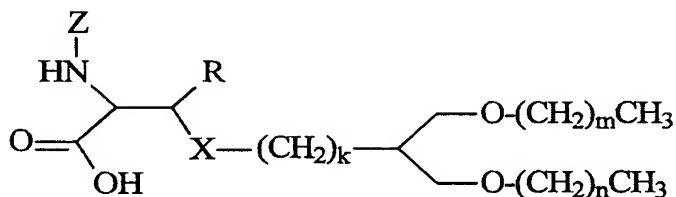
wherein Z is H or an amino protecting group,

R is H or CH₃, and

n is an integer with values ranging from 6 to 26.

10

83. A lipidated amino acid of claim 1 is of the following composition:



15

wherein,

X is -O- or -S-,

Z is H or an amino protecting group,

R is H or CH₃,

k is an integer with values ranging from 0 to 6

20

m and n are independent integers with values ranging from 6 to 26.

84. The lipidated amino acid of claims 82 or 83 where Z is Boc or Fmoc.

85. The lipidated amino acid of claims 82 or 83 where Z is H.

84. The use of the lipidated amino acid of any one of claims 82-84 for the purpose of specific modulation of immune responses to an antigen.

85. The glycopeptide of claim 1 which comprises the amino acid sequence PDTRP (AAs 6-10 of SEQ ID NO:10).

86. The glycolipopeptide of claim 85 which comprises the amino acid sequence SAPDTRP (AAs 4-10 of SEQ ID NO:10).

87. The glycolipopeptide of claim 1 which comprises at least one copy of (a) the MUC1 consensus tandem repeat

GVTSAPDTRPAPGSTAPPAH (SEQ ID NO:10) ,

(b) a cyclic permutation thereof, or (c) a sequence substantially identical to (a) or (b) above.

88. The glycolipopeptide of claim 87 which comprises at least two copies of (a), (b) or (c).

89. The glycolipopeptide of claim 1 which comprises a tumor-associated carbohydrate epitope.

90. The glycolipopeptide of claim 89 where the carbohydrate epitope is GalNAc (Tn).

91. The glycolipopeptide of claim 89 where the carbohydrate epitope is sialyl Tn.

92. The glycolipopeptide of claim 89 where the carbohydrate epitope is Gal-GalNAc (TF).

93. The glycolipopeptide of claim 85 where the threonine of PDTRP is glycosylated.

94. The glycolipopeptide of claim 93 where the threonine of PDTRP is O-linked to Tn.

5 95. The glycopeptide of claim 1 in which at least two amino acids are lipidated.

96. The glycolipopeptide claim 1 in which at least two interior amino acids are lipidated.

10 97. The glycolipopeptide of claim 1 in which all of the lipidated amino acids are interior amino acids.

98. The glycolipopeptide of claim 1 characterized by a carboxy terminal sequence SSL, where each of the serines is lipidated.

15 99. The glycopeptide of claim 1 in which at least one strongly lipophilic group of at least one lipidated amino acid has a logP, as predicted by the Meylan algorithm of at least 2.7.

100. The glycopeptide of claim 99 where said predicted logP is at least 3.

20 101. The glycopeptide of claim 99 where said predicted logP is at least 4.

102. The glycopeptide of claim 99 where said predicted logP is at least 5.

25 103. The glycopeptide of claim 99 where said predicted logP is at least 6.

104. The glycopeptide of claim 99 where said predicted logP is at least 7.

105. The glycopeptide of claim 99 where said predicted logP is at least 8.

30 106. The glycopeptide of claim 99 where said predicted logP is at least 9.

107. The glycopeptide of claim 99 where said predicted logP is at least 10.

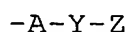
108. The glycolipopeptide claim 1 in which the amino terminal amino acid comprises a strongly lipophilic group.

5 109. The glycolipopeptide of claim 1 in which the carboxy terminal amino acid comprises a strongly lipophilic group.

10 110. The glycolipopeptide of claim 1 in which at least one lipidated amino acid is selected from the group consisting of lipidated Ser, Thr, Asp, Glu, Cys, Tyr, Lys, Arg, Asn or Gln.

111. The glycolipopeptide of claim 110 where the lipidated amino acid is lipidated Ser or Thr.

15 112. The glycolipopeptide of claim 1 where at least one lipidated amino acid comprises a side chain of the formula



where A is optional but, if present, is an organic group of not more than 12 atoms other than hydrogen;

20 Y is a spacer of not more than 12 atoms other than hydrogen, and comprising nitrogen, oxygen, sulfur or phosphorous, and Z is a strongly lipophilic group.

113. The glycolipopeptide of claim 112 in which A, if present, is an alkyl of 1-4 carbon atoms.

25 114. The glycolipopeptide of claim 113 in which A is present and is $-\text{CH}_2-$ or $-\text{CH}(\text{CH}_3)-$.

115. The glycolipopeptide of claims 112 in which Y comprises a group selected from the group consisting of $-\text{O}-$, $-\text{S}-$, $-\text{NH}-$, $-\text{NR}-$, $-\text{PO}_4-$, $-\text{C}(=\text{O})-$, $-\text{C}(=\text{S})-$, $-\text{C}(=\text{NH})-$, and $-\text{C}(=\text{NR})-$, where R is 1-4 alkyl.

30 116. The glycolipopeptide of claim 115 in which Y is $-\text{NHCO}-$, $-\text{OCO}-$ or $-\text{SCO}-$.

117. The glycolipopeptide of claim 115 in which Y is -CONH- or -CH₂NH-.

118. The glycolipopeptide of claim 115 in which Y is -O-, -S- or -NH-.

5 119. The glycolipopeptide of claim 112 in which Z is at least partially aromatic.

120. The glycolipopeptide of claim 112 in which Z is aliphatic.

10 121. The glycolipopeptide of claim 112 in which Z comprises at least one moiety of the form -A'-Y'-Z', where A', Y' and Z' are defined analogously to A, Y and Z, respectively.

122. The glycolipopeptide of claim 121 where Y' is -O- and Z' is an alkyl group.

15 123. The glycolipopeptide of claim 122 where A is -(CH₂)_i-, where i is 0 or 1, or Z' is -(CH₂)_jCH₃, where j is 6 to 26.

20 124. The glycolipopeptide of claim 112 in which Z comprises -B(-Y'-Z')_n, in which B is a branched organic group of not more than 12 atoms than hydrogen, each Y' is an independently chosen spacer of not more than 12 atoms other than hydrogen, and comprising nitrogen, oxygen, sulfur or phosphorous, and each Z' is an independently chosen strongly lipophilic group, and n is at least two.

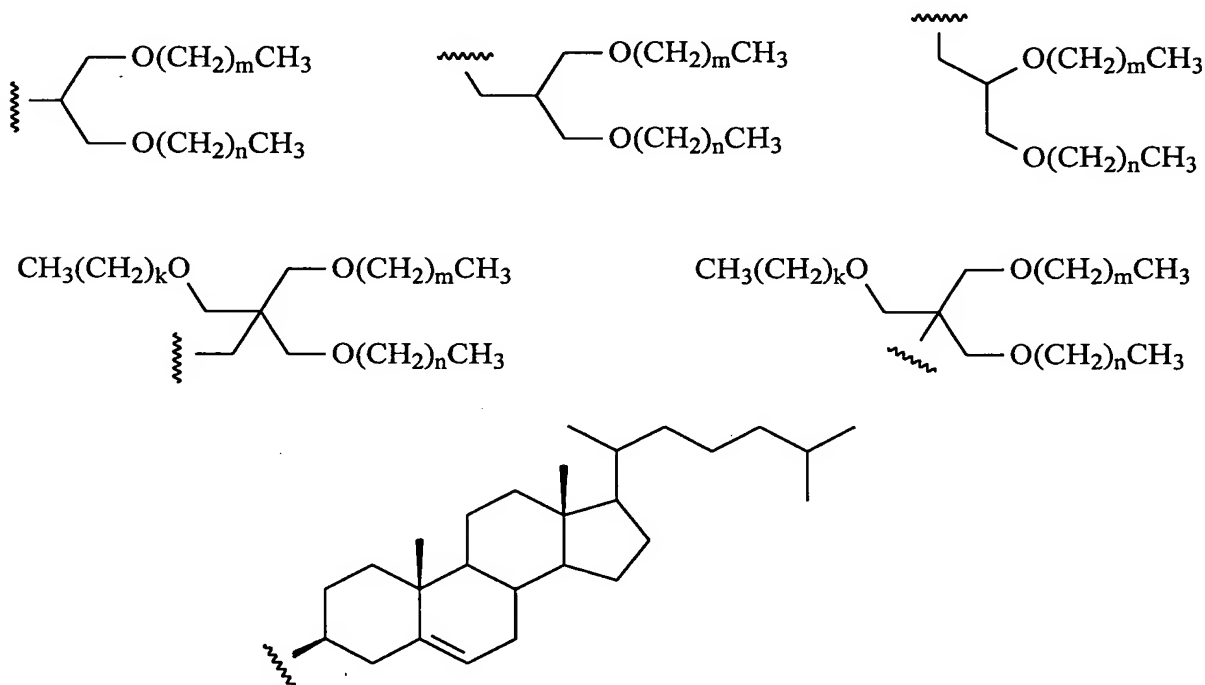
25 125. The glycolipopeptide of claim 124 in which n is 2 or 3.

126. The glycolipopeptide of claim 124 in which each Y' is -O- and each Z' independently is -(CH₂)_jCH₃, where j=6 to 26.

30 127. The glycolipopeptide of claim 124 in which n=2 and B is -CH(CH₂-)₂.

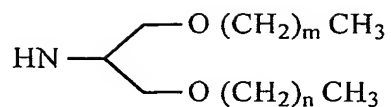
128. The glycolipopeptide of claim 124 in which $n=2$ and B is $-\text{C}(\text{CH}_2-)_3$.

129. The glycolipopeptide of claim 1 where the strongly lipophilic group of at least one lipidated amino acid comprises at least one of the following structures:



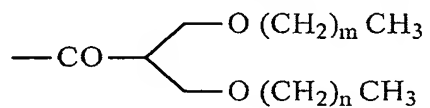
where m , n , and k are independent integers with values ranging from 3 to 30.

130. The glycolipopeptide of claim 1 wherein a strongly lipophilic group of at least one lipidated amino acid comprises the structure



where m and n are independent integers with values ranging from 6 to 26.

131. The glycolipopeptide of any one of claims 1-7
 5 wherein a strongly lipophilic group of at least one
 lipidated amino acid comprises the structure



where m and n are independently chosen integers 6 to 26.